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(57) Abstract

An oral composition for fish or similar aquatic creatures comprises a stable viscous water-in-oil emulsion containing a vaccine against fish disease, the emulsion being carried on fish feed particles. The oral composition can be administered to fish in a non-labour-intensive manner and causes no stress to the fish, in complete contrast to conventional vaccine administration by parenteral injection. Moreover, the oral composition can substantially reduce the minimum effective vaccine dose.

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VACCINE COMPOSITIONS FOR FISH

This invention relates to compositions useful for oral administration of sensitive materials such as vaccines to aquatic animals, especially fish.

The intensive rearing of fish such as salmon, which today is practised on a wide scale, suffers from the disadvantage that the entire stock of fish in a facility may become infected with disease. The commercial consequences of a serious disease outbreak can be enormous. Several well-recognised fish diseases are causing severe problems in the fish farming industry. Examples are bacterial kidney disease, caused by Renibacterium salmoninarum; enteric redmouth, caused by Yersinia ruckeri; and vibriosis, caused by various strains of Vibrio, notably V.anguillarum and V.salmonicida. Currently, the most significant disease, at least as far as the farming in Northern Europe of Atlantic salmon (Salmo salar) is concerned, is furunculosis; this is caused by the bacterium Aeromonas salmonicida.

The desirability of immunising farmed fish against such diseases has long been recognised. However, technical progress in this area has been slow. This applies both to the effectiveness of the commercially available vaccines (a wholly effective vaccine for salmon against furunculosis has yet to be developed), and also to the means by which such vaccines are administered to the fish. Traditionally, administration of vaccines to fish has either been by immersion (which is wasteful as well as of limited efficacy), or by injection. Although injection of individual fish provides a sure way of delivering a vaccine, it suffers from the disadvantages that it is very labour-intensive, and moreover the handling and injection cause considerable stress to the fish, and can precipitate disease problems or at least cause temporarily retarded growth. Usually fish are anaesthetised prior to injection.

There is a clear commercial need for a vaccine delivery system for fish which is less labour-intensive, and which causes little or no stress to the fish.

For some while it has been recognised that an oral delivery system, in which a vaccine is administered to the fish either as part of the regular diet or in a composition administered together with the regular diet, would be beneficial. Reported experiments involving attempted oral administration of fish vaccines have yielded inconsistent results, and no effective oral vaccine has yet become available commercially. Particular problems recognised in the oral route are: possible loss of or damage to essential vaccine components during manufacture of the composition; possible loss of water-soluble vaccine components in the aqueous environment in which the fish live; and possible

degradation of the vaccine within the intestine of the fish before the vaccine has induced a protective response.

By the present invention we provide a particulate composition for oral administration to aquatic creatures, especially fish, comprising a water-in-oil emulsion containing a sensitive agent such as a vaccine or the like, the emulsion being carried on a solid edible carrier material, preferably particles of a feed-stuff appropriate for the aquatic creatures.

The skilled reader will readily appreciate that the invention can be adapted for use with aquatic creatures other than true fish, for example crustacea such as prawns, shrimps, lobsters and crabs, and molluscs such as oysters. For convenience, the invention will be described in relation to fish, and the term "fish" should be understood as encompassing other aquatic life forms that may benefit from the oral administration of vaccines and the like. Examples of true fish that are reared on a substantial scale in captivity in various parts of the world are: Atlantic salmon, Pacific salmon, rainbow trout, brown trout, catfish, halibut, turbot, carp and tilapia.

Generally, the sensitive agent will be water-soluble rather than oil-soluble, and hence dispersed in the aqueous phase of the emulsion. The emulsion should contain sufficient of the sensitive agent to provide the desired effect, eg. an effective immune response, when the composition is administered to the fish.

Preferably the emulsion is applied to particulate fish feed that is nutritionally deficient in oil.

Preferably the emulsion comprises a water:oil mixture containing not more than about 70% by weight water. More preferably, the water content is not greater than about 65% by weight. Nevertheless, the emulsion must contain sufficient water to act as a carrier for the water-soluble agent, and a particularly preferred water:oil weight ratio range is 6:4 to 4:6.

Preferably the emulsion comprises at least about 1% by weight of the final composition. More preferably, the emulsion comprises at least about 2%, and ideally at least about 3%, by weight of the composition. Generally, the amount of emulsion is not greater than about 10% by weight.

The emulsion should have a viscous consistency, ie. creamy and flowable, to enable it to be applied uniformly to the particulate carrier.

The carrier should be relatively dry and non-oily so that the applied emulsion can be absorbed, at least partially, by the particles. Preferably the particulate carrier is un-oiled feed in granular or pelletted form. The composition of the feed is not critical to the invention, as long as the formulation is appropriate for the fish to which the composition is to be fed, and at the time of adding the emulsion the particles do not contain oil or other fluid ingredients in such amounts that the emulsion cannot be absorbed by the particles. After the application of the emulsion, the particles should still be sufficiently dry and free-flowing to be handled in the same manner as conventional fish feed. Typical fish feeds are high in protein, and are conventionally based on fish meal with added components such as cereals, and oil as an energy source. Fish meal naturally contains a certain

amount of oil, but usually this needs to be boosted for nutritional reasons. The additional oil is usually added to the granules or pellets after these have been formed; the oil-deficient material is herein termed "un-oiled". The size of fish feed pellets varies widely, depending on the type and age of the fish; for salmon the pellets typically have a diameter from about 1 to about 6mm, with pellets of about 3mm being appropriate at the smolt stage. Fish feeds can also contain minor components such as vitamins, minerals, preservatives and pigments. Alternatively, non-feed carriers can be used, but these should provide final compositions that are perceived by the fish as normal feed, otherwise the fish may ignore or reject the composition. Fish tend to be very critical in accepting feed particles, and many factors such as size, shape, colour and density, which all affect the "behaviour" of the particles in water, can be very influential. A composition of the invention should therefore be made to have physical properties as similar as possible to those of the normal feed to which the fish are accustomed.

The emulsion should be sufficiently stable to protect the aqueous phase for a time sufficient to enable the composition to be produced and administered to the fish. In general, the emulsion should not "crack", ie. separate into distinct aqueous and oil phases, in less than one week. Preferably, the emulsion is stable for at least one month.

The oil is preferably a neutral oil, because the presence of free fatty acids can sometimes interfere with the formation of an adequately stable emulsion. Preferably, the level of free fatty acids should be not greater than about 5% by weight of the oil, and ideally

not greater than about 3%. Preferred oils are whole fish body oil and neutral marine oil. If desired, the emulsion can incorporate an antioxidant such as butylated hydroxytoluene or ethoxyquin.

The emulsifier should be food grade, and is preferably a lecithin. An ideal emulsifier is soya lysolecithin (a modified phospholipid) and examples are available commercially from Unimills BV under the trade name "Bolek". Generally, the emulsifier will comprise from about 0.1 to about 5% by weight of the total emulsion.

The emulsion can be prepared by blending the oil and aqueous phases, generally at ambient temperature, using a homogeniser. In-line homogenising equipment is preferred. Preferably the components can be recycled two or more times through the homogeniser if desired.

The invention also provides a process for the preparation of an orally administrable composition for fish, wherein a stable water-in-oil emulsion containing a sensitive agent such as a vaccine, is applied to a particulate feedstuff, preferably by pan-coating or the like.

In a preferred embodiment of the invention, additional oil, eg. from about 1 to about 3% by weight, is applied to the particulate composition after the application thereto of the emulsion. This can also be achieved by pan-coating.

An important aspect of the invention is the use of an oral delivery system to reduce the total effective administered dose level of a vaccine that would otherwise

need to be administered by a non-oral route (generally by injection or immersion) in a substantially higher total dose. We have found that administration of a vaccine in a composition according to the invention surprisingly enables an effective protective response in fish to be achieved at a much lower dose level. In some circumstances, a 10-fold reduction in the effective dose level can be achieved.

For example, we have conducted trials with a commercially-available furunculosis vaccine ("Furogen") which is recommended for single administration by parenteral injection at a dose level of 0.1 ml per fish. We have administered this commercially-available vaccine orally to salmon using a composition of the invention. We have found that a total dose level of only 0.001 ml "Furogen" per fish, administered orally over a ten-day period, provided protection at least as effective as the single recommended 0.1 ml dose administered by injection. In contrast to the injection route, oral administration of the vaccine in accordance with the invention did not cause any stress to the fish, and could be conducted as part of their normal feeding regime.

Although the theory behind the effectiveness of the invention is not yet fully understood, we believe that the oral administration of a furunculosis vaccine in accordance with the invention promotes a cellular response in the fish, which leads to enhanced protection. This protection seems unrelated to the quantity of antibodies within the circulation system of the fish. Administration of the same vaccine by injection may lead to enhanced circulating antibody levels, but apparently its impact at the cellular level has not been studied.

We believe that the oil in a composition of the invention protects the antigenic components of the vaccine from degradation in the upper acidic regions of the gut of the fish, while allowing the antigenic components to be released in the lower alkaline regions where the oil is digested enzymatically.

An oral composition of the invention, containing a vaccine, can be used in place of conventional injectable and/or immersion vaccines. Alternatively, a combination of routes of administration can be employed, for example by using the oral route to provide a basic level of protection which can be boosted at an appropriate occasion (eg. when fish are moved or counted, or at a time of greater perceived infection risk) by a supplementary injection or immersion.

Although it is envisaged that a composition of the invention will generally be administered to farmed fish, it can also be distributed "in the wild" to reduce the incidence of fish disease in the natural environment, so benefiting the indigenous fish population and enhancing global fish resources.

The oral composition of the invention can be prepared "on the spot" for immediate administration to fish. Alternatively, a composition of the invention can be prepared and packaged in any manner conventionally used for commercially-available fish feeds, for example in grease-proof sacks, and supplied as a commercial product. The shelf-life of the composition can be enhanced, if necessary, by the incorporation of preservatives, eg. in the applied emulsion.

The invention can be used to administer any fish vaccine that can induce an immune response via the gut. For example, the vaccine can be a simple bacterin composition, ie. a killed whole culture of an infective organism, or an extract of a killed culture. The vaccine can comprise toxoided components, ie. toxic factors associated with the infective organism which have been treated, eg. by chemical means such as formaldehyde or glutaraldehyde treatment, to reduce their toxic effect without seriously impairing their antigenic properties. Where appropriate, the vaccine can comprise live organisms, preferably attenuated, eg. by controlled culture or by genetic manipulation. The active components of the vaccine can include, or indeed can consist entirely of, factors originally identified in the disease-causing organism but subsequently produced for the purpose of the vaccine by expression in genetically-modified organisms such as E.coli.

By way of example only, a composition in accordance with the invention can be made as follows.

Example 1

An approximately 1:1 mixture of whole fish body oil and diluted aqueous "Furogen" injectable vaccine (an aqueous composition containing a bacterin derived from killed Aeromonas organisms) was blended by multiple passes through an ultrasonic homogeniser, to form a stable creamy water-in-oil emulsion, with the aid of 3% (total weight) of "Bolek K" lecithin emulsifier. "Bolek K" may no longer be commercially available, but other lecithin emulsifiers from the "Bolek" range, such as "Bolek M", can be regarded as identical for the practical purposes of this invention. "Furogen" is available commercially from Aquahealth

Limited, Canada and supplied in two formulations: for parenteral injection and for immersion.

The emulsion was soaked into un-oiled conventional extruded 3mm diameter fish feed pellets (based on fish meal and cereal), by pan-coating, using 5% emulsion by weight of the feed. An additional 2.5% fish oil by weight was added subsequently to the feed by the same method.

Fish normally consume 1-3% body weight/day, and the quantity of "Furogen" was adjusted, by dilution with water, to provide a total of about 0.001 ml of the commercially-supplied injectable composition over a 10-day trickle feed period.

The resulting composition was physically indistinguishable from conventional oiled fish feed pellets, and could be handled and fed to fish in any conventional manner, eg. by hand or by mechanical feeders.

Example 2

Trials in Atlantic salmon (Salmo salar) were conducted as follows, using oral compositions made as in Example 1, with a control group of fish receiving the commercially-available injectable composition.

Groups and treatment

All fish were 1990 smolts from a commercial salmon hatchery.

a) 1000 fish; composition of Example 1 containing "Furogen" immersion vaccine formulation @ 0.1 ml/fish*.

b) 1000 fish; composition of Example 1 containing "Furogen" injectable vaccine formulation @ 0.1 ml/fish*.

c) 1000 fish; composition of Example 1 containing "Furogen" immersion vaccine formulation @ 0.01 ml/fish*.

d) 1000 fish; composition of Example 1 containing "Furogen" immersion vaccine formulation @ 0.001 ml/fish*.

Control: 750 fish receiving a single dose of "Furogen" by injection @ 0.1 ml/fish.

*Total received on average per fish over a 10-day feeding period.

Method

To identify the groups, all fish were marked using a panjetter and Alcian blue dye (4%) before the beginning of the experiment.

Fish were fed their respective vaccines over a 10-day period in separate tanks, with a common water supply.

The fish were sample weighed and cultured for A.salmonicida. Low-level infection was already present, representing a typical on-farm situation. Further challenge was natural, from the local water supply.

The results are shown in Table 1, and indicate the percentage cumulative mortality of the trial groups over a one-month period commencing from the start of vaccination.

These results demonstrate that the oral route is an effective delivery system, and produces results at least as good as the injection route. Indeed, the results indicate that the oral route can enable lower administered vaccine levels to be used and at the same time induce enhanced protection.

Table 1

Composition	Dose (ml/fish)	Mortality (%)
Control	0.1	15.0
a)	0.1	12.6
b)	0.1	7.0
c)	0.01	12.8
d)	0.001	1.4

CLAIMS

1. A composition for oral administration to aquatic creatures, especially fish, comprising a water-in-oil emulsion containing a sensitive agent such as a vaccine or the like, the emulsion being carried on a solid edible particulate carrier material, preferably particles of a feedstuff appropriate for the aquatic creatures.

2. A composition according to claim 1, wherein the emulsion is carried on a particulate fish feed that is nutritionally deficient in oil.

3. A process for the preparation of an orally administrable composition for aquatic creatures, especially fish, wherein a water-in-oil emulsion containing a sensitive agent, such as a vaccine or the like, is applied to a particulate feedstuff.

4. A process according to claim 4, wherein additional oil is applied to the particulate product after the application thereto of the emulsion.

5. A composition or process according to any one of the preceding claims, wherein the sensitive agent is a vaccine against furunculosis.

6. A composition or process according to any one of the preceding claims, wherein the emulsion comprises a water:oil mixture of about 6:4 to 4:6 by weight.

7. A composition or process according to any one of the preceding claims, wherein the emulsion comprises at least about 1% by weight of the final composition.

8. Use of a composition according to any one of claims 1, 2, 5, 6 or 7 to reduce the total effective administered dose level of a vaccine that would otherwise need to be administered by a non-oral route (generally by injection or immersion) in a substantially higher total dose.

9. Use of an oral delivery system to enhance the protective response induced by, and/or reduce the effective dose level of, a vaccine administered to aquatic creatures, especially fish.

10. A method of vaccinating fish involving oral administration of a composition according to any one of claims 1, 2, 5, 6 or 7.